XENOTRANSPLANTATION

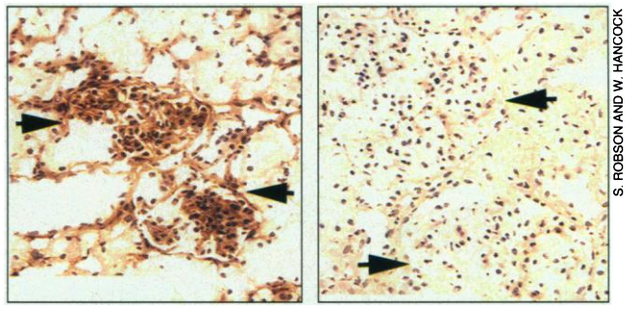
New Ways to Avoid Organ Rejection Buoy Hopes

BOSTON—The body's first reaction to truly foreign tissue is not a subtle one. Organs transplanted from pigs into nonhuman primates often swell and blacken within minutes, as blood cells called platelets rush in and seize on the inner walls of blood vessels, clumping together to strangle blood flow. This gruesome phenomenon, called hyperacute rejection, is one of the biggest roadblocks obstructing what many see as a route to alleviating the dire shortage of human donor organs—animal-to-human organ transplants (*Science*, 18 November 1994, p. 1148).

Now researchers may have found ways to help keep this route—and the blood vessels—open. At the Third International Congress on Xenotransplantation in Boston last week, scientists presented research pointing to two different strategies. One group reported that when host blood rushes into newly transplanted tissue, reactive oxygen molecules in the blood may damage a key platelet-inhibiting enzyme called ecto-ATPase. Treating this tissue with antioxidant drugs prophylactically keeps the enzyme active, say Simon Robson, a hepatologist (specializing in the study of the liver) at New England Deaconess Hospital (NEDH) in Boston, and his colleagues, immunologist Fritz Bach, surgeon Daniel Candinas, and pathologist Wayne Hancock.

Another group of researchers described a different strategy that focuses on genetics. Immunologist David White, a founder of the British transplant company Imutran, gave the first detailed accounts of his experiments, announced in a press conference 3 weeks ago, showing that donor pig organs genetically engineered to express human immune regulators have survived in monkeys for up to 8 weeks with no signs of rejection. By inhibiting the activation of key immune proteins called complement, White says, this approach makes hyperacute rejection "a thing of the past."

The NEDH research drew a largely positive response, because it implies that hyperacute rejection could be controlled without suppressing the host's immune system, which also contributes to rejection. "It could be very important work," says immunologist David Sachs, director of the Transplantation Biology Research Center at Massachusetts General Hospital (MGH) and editor of the journal *Xenotransplantation*. "With a specific way of inhibiting [hyperacute rejection] that doesn't require a lot of immunosuppression, we'll be much better off." Participants took a more cautious view of White's work, noting that natural variations in immune response in transgenic animals could help account for enhanced survival. Still, conference attendees were heartened by these signs of progress. "One by one we're going to overcome the major obstacles," says immunologist Megan Sykes of MGH.



S. ROBSON AND W. HANCOCK

Signs of rejection. (Left) Normal pig tissue, tagged with an antibody for the enzyme ecto-ATPase *(red)*, has a lot of the enzyme, which prevents blood vessel clotting. **(Right)** In pig tissue transplanted into baboons—and quickly rejected—the enzyme has been degraded.

Both hyperacute rejection and a related phenomenon that follows within days or weeks, called delayed xenograft rejection, are triggered at the surface of the endothelial cells that line blood vessels. Under normal conditions, these cells help keep blood in a liquid state by producing proteins and surface enzymes that inhibit the aggregation of platelets. But they fail to do so where host blood meets the vessel walls in a donor organ. Researchers have pinned much of the blame on immune system warriors such as xenoreactive natural antibodies (XNAs) and complement proteins, which cause endothelial cells to shrivel, exposing platelet-activating molecules in the tissues below.

But studies over the last several years by Robson, Bach, and colleagues at NEDH indicated that injured endothelial cells undergo changes that actually accelerate clotting without involving the immune system. Robson exposed cultured pig endothelial cells to inflammatory mediators and discovered that the cells lost their usual ability to inhibit clotting.

At last week's conference, Robson provided an explanation for this lost ability: The enzyme ecto-ATPase, which inhibits platelet aggregation, was missing or inactivated on the injured cells. He had exposed cultured pig endothelial cells to inflammatory mediators such as reactive oxygen intermediates, and within 30 minutes, ecto-ATPase activity dropped by more than 50%. And because the inflammatory agents Robson used were rich in oxygen, he fingered the element as the ecto-ATPase destroyer.

White blood cells called neutrophils generate highly reactive oxygen radicals, which underlie many forms of inflammation. So Robson and his colleagues speculated that oxygen-generating neutrophils in blood rushing into a newly transplanted organ can change the shape of ecto-ATPase, rendering it inactive or making it more likely to be sheared off.

Indeed, Robson found that when he treated endothelial cells with antioxidant drugs such as catalase and superoxide dismutase before exposure to inflammatory agents, they didn't lose their ecto-ATPase. Such drugs might be used to stabilize ecto-ATPase in the organs of donor animals before transplantation. Bach also raises the possibility of inserting the gene encoding human ecto-ATPase into pig embryos to produce animals that express the enzyme in greater amounts or in a more oxidation-resistant form.

Other researchers applaud the work because it underscores warnings that therapies directed at eliminating immune responses to transplantation may not be enough to control hyperacute rejection. "It may not be enough to focus on inhibiting antibodies and complement. Regulation of clotting and coagulation may also be very important," says MGH's Sykes. "That's why what Robson is doing is very interesting."

At least one researcher, however, was not at all interested in modifying endothelial cell responses. "What my data say is, you don't need it," says Imutran's White. Clotting simply doesn't occur in hearts transplanted into cynomolgus monkeys from Imutran's genetically modified pigs, he contends.

In Imutran's recent experiment, detailed at the congress to the intense interest of participants, researchers placed one copy of the gene encoding a human complement-regulating protein, decay accelerating factor (DAF), into pig embryos. Adult pigs then expressed DAF in tissues and endothelial cells at three to four times human levels. Without immunosuppressive drugs, monkeys receiving hearts from these transgenic pigs survived for a median of 5.1 days, compared to 1.6 days for controls receiving nontransgenic hearts. And with immunosuppression, the monkeys receiving transgenic hearts survived for a median of 40 days, weeks longer than previously achieved in a cross-species transplant.

"With immunosuppression, we can get long-term survival," White says. "I can envisage a scenario where, 12 months from now, our data will have achieved the 'comfort factor' where we think we are justified in going to clinical trials in humans." DAF in the transgenic hearts may inhibit complement proteins from attacking endothelial cells, so that platelets never come into contact with subendothelial clotting factors.

Other researchers at the congress praised Imutran's advance, but called White's timetable overoptimistic. "This work potentially makes an important contribution, but it certainly does not indicate to me that we are ready to use these transgenic hearts clinically," says MGH's Sachs. "We still have to worry about delayed rejection and [long-term] T cell immunity." Sachs is also concerned because some of the donor pigs had unexpectedly low levels of an antigen that binds to XNAs. This could have helped avoid hyperacute rejection. White responds that data on antigen levels are "not robust," and the contrast between healthy transgenic hearts and unhealthy control hearts is "amazing."

Many researchers believe that only a combination of donor- and recipient-based therapies, both pharmaceutical and genetic, will eventually make xenotransplantation routine. "This is a big problem, and people are going to have to work together to solve it," says Sachs. "The exciting thing is that every step looks as if it is amenable to being dealt with. I believe it eventually will work."

–Wade Roush

ATMOSPHERIC RESEARCH

Lofty Flashes Come Down to Earth

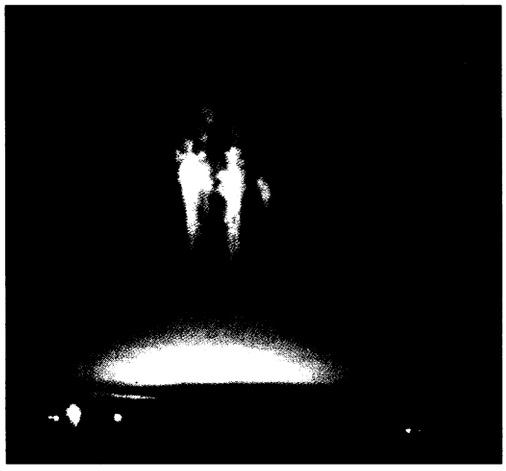
Walter Lyons had a mystery right in his backyard, which lies at the foot of the Rocky Mountains and commands a view over the Great Plains. So last summer he threw a little garden party, inviting over a few friends and colleagues—16 research groups in all, with their instruments—to help him solve it. The mystery was what could be creating the menagerie of exotic flashes that can be detected from his yard as they light up the atmosphere high above giant thunderstorms on the plains. And last summer's gathering at Lyons's home on Yucca Ridge near Fort Collins, Colorado, along with research efforts from other vantage points, has gone a long way toward providing an answer.

"There's so much data it's almost overwhelming," says Lyons, a meteorologist at Mission Research Corporation's ASTER Division in Fort Collins. Most of the observations are still being digested, but they are already showing how exceptional lightning strokes that drain storm clouds of huge amounts of electrical charge could spark the flashes. By analyzing spectra of the flashes and measurements of their timing, researchers have traced two different mechanisms for setting the upper atmosphere aglow, one driven by a quick pulse of energy from the lightning stroke and the other by a slower change in the atmosphere's electric field.

A year ago, observations from Lyons's yard and elsewhere had shown that the flashes come in forms ranging from carrot-shaped "red sprites" at altitudes of 40 to 90 kilometers to a fainter, broader glow at still higher altitudes (*Science*, 5 August 1994, p. 740). Analyzing the data, Dennis Boccippio and Earle Williams of the Massachusetts Institute of Technology and Lyons found that the sprites, at least, coincide with the huge, positively charged cloud-to-ground lightning bolts from the biggest thunderstorms (*Science*, 25 August, p. 1088). Although that pointed to lightning as the sprites' ultimate cause, researchers did not know the mechanism.

The colors of the sprites offered a clue, however. Rare visual sightings and color video images of these brief flashes had shown that they have a red hue reminiscent of the aurora. That suggested that the light of a sprite, like that of the aurora, comes from oxygen or nitrogen molecules excited by collisions with high-energy electrons. Now two groups—Stephen Mende of Lockheed Palo Alto Research Laboratory in California and his colleagues working at the Fort Collins site and Davis Sentman and colleagues at the University of Alaska, who made their observations from the top of Colorado's Mount Evans—have recorded spectra of sprite light and found that its source is molecular nitrogen excited by electron collisions.

But how could lightning only 5 to 10 kilometers above the ground unleash electrons at altitudes 10 times higher? One possibility relied on the 100-microsecond pulse of electromagnetic energy broadcast by a lightning stroke. Moving upward at the speed of light into thinner regions of the atmosphere, it might eventually be able to rip electrons from atmospheric molecules and accelerate them unhindered by too many collisions with other molecules.



S. B. MENDE AND R. L. RAIRDEN

High lights. Changes in the atmosphere's electric field above a thunderstorm spawned these sprites.

Researchers had also considered a slower, "electrostatic" mechanism that depends on the charge a cloud induces in the atmosphere above it as it electrifies before unleashing a lightning stroke. The cloud's own charge is annihilated when the lightning suddenly transfers upwards of 1000 coulombs of charge to the ground, while the field above the cloud lingers. And because the field is no longer nullified by the cloud charge, for a few milliseconds it's strong enough to ionize molecules and accelerate the resulting electrons.

Measurements of the precise timing of lightning strokes and flashes now imply that both mechanisms may be at work—in different parts of the upper atmosphere. Mende and his colleagues found that sprites lag the huge positive lightning strokes triggering them by several milliseconds. That's much longer than it would take for the electromagnetic pulse from a lightning stroke to arrive at the altitude of a sprite. But the timing is about right for the slower electrostatic mechanism, notes Williams.

Electromagnetic pulses could still have a role, however, in the fainter, broader flashes sometimes recorded at around 90 kilometers in the lowermost ionosphere. Working in Lyons's yard, Hiroshi Fukunishi of Tohoku University and his colleagues found that these flashes appeared less than 1 microsecond after the lightning—about the right interval for an electromagnetic pulse to reach the ionosphere. The finding also fits theoretical calculations made by Umran Inan and his colleagues at Stanford University, who had pointed out that a pulse was far more likely to trigger a flash in the ionosphere, where molecules are already ionized, than at the lower altitudes where sprites appear.

Although researchers have tentatively sorted out mechanisms for two upper-atmosphere phenomena, many questions remain. Theorists have yet to publish even a hand-waving explanation of yet another species of flash, the "blue jets" that explode from storm-cloud tops. And the menagerie of middle-atmosphere flashes may contain even more strange beasts. There will be plenty more work for the next yard party on Yucca Ridge.

–Richard A. Kerr